

SUPPLEMENTAL METHODS

Generation of GATA5 mutant mice

The 5'-arm, KO arm (exons 3-6) and 3'-arm of the *Gata5* genomic locus were isolated from the bMQ221g13 BAC clone (1). A 5.9-Kb genomic DNA fragment (5'-arm) that included exons 1 and 2 with flanking introns was digested with *Avr2* restriction enzyme and subcloned upstream of the LoxP site of the targeting vector. A 3.8-Kb fragment (the deleted region) that encoded exons 3-6 was digested with *Pac1* and *Nhe1* restriction enzymes and inserted in between the LoxP sites. A 4-Kb fragment (3'-arm) was digested with *Sal1* and *Kpn1* restriction enzymes and subcloned downstream of the LoxP site in the targeting vector.

The targeting vector was digested with *Not1* and *Kpn1* and electroporated into 129Sv embryonic stem (ES) cells. Following negative selection with G-418, 400 individual ES cell clones were isolated and analyzed for homologous recombination by southern blotting. Two clones with a properly targeted *Gata5* allele were microinjected into 3.5-d C57BL/6 blastocysts, which were implanted into CD1 pseudopregnant females. The resulting chimeras were bred to C57BL/6 mice to achieve germline transmission. To obtain mice with a *Gata5*-null allele, *Gata5*^{WT/neo-loxP} mice were crossed with CMV-Cre mice to generate *Gata5*^{+/-} mice, which were then intercrossed together to obtain *Gata5*^{-/-} mice. The *Gata5* null mice were maintained in a mixed 129SV/C57BL/6 background and analyzed between generations F3 to F5.

A similar breeding strategy was used to generate mutant mice lacking *Gata5* in the endocardium. Of note, *Gata5*^{WT/neo-loxP} mice were crossed with *ACTB1-Flpe* mice to generate *Gata5*^{+/-loxP} mice, which were then crossed with *Tie2-cre* transgenic mice to obtain *Tie2-cre;Gata5*^{+/-loxP} mice. *Tie2-cre;Gata5*^{+/-loxP} mice were then intercrossed together to obtain *Tie2-cre;Gata5*^{+/+}, *Tie2-cre;Gata5*^{+/-loxP} and *Tie2-cre;Gata5*^{Flox/Flox} mice. *Tie2-cre;Gata5*^{Flox/Flox} mice were kept in a mixed 129SV/C57BL/6 background and analyzed between generations F2 to F5.

Tail genomic DNA was digested with *Sca1* or *Drd1* and analyzed using a standard Southern blot protocol with the probes indicated in Figure 1.

SUPPLEMENTAL REFERENCE

1. Adams DJ, Quail MA, Cox T et al. A genome-wide, end-sequenced 129Sv BAC library resource for targeting vector construction. *Genomics*. 2005;86(6):753-758.

Supplemental Table1

Summary of Q-PCR primers

Gene	Primer sequence	Accession number	Position
<i>ANF</i>	5'-TGCCGGTAGAAGATGAGGTC-3'	XM_131840	Exon 2
	5'-AGCAGCTGGATCTTCGTAGG-3'		
<i>Bmp4</i>	5'-GGGAACTTTTCGATGTGAGC-3'	NM_007554.2	Exon 4
	5'-ATGCTTGGGACTACGTTTGG-3'		
<i>BNP</i>	5'-CAGCTCTTGAAGGACCAAGG-3'	NM_008726	Exon 2-3
	5'-AGAGACCCAGGCAGAGTCAG-3'		
<i>Cadherin 5</i> (<i>VE-cadherin</i>)	5'-TGCACATCTACGGCTACGAG-3'	NM_009868.4	Exons 12/3'-UTR
	5'-CTGATCCAGGTTGCAATGAG-3'		
<i>EphB4</i>	5'-TCTGCTTTCGGTTCTGTGG-3'	NM_001159571.1	Exons 16-17
	5'-GGTGAGTCCTTGGAGGTCAG-3'		
<i>ErbB2</i>	5'-ATGGACAGCACCTTCTACCG-3'	NM_001003817.1	Exons 25-26
	5'-GGTCACCATCAAACACATCG-3'		
<i>Gata4</i>	5'-CAGCAGCAGTGAAGAGATGC-3'	NM_144730.1	C-terminal
	5'-ATGTCCCCATGACTGTCAGC-3'		
<i>Gata5</i>	5'-ACATGAGTTCCGACGTAGCC-3'	NM_008093.1	Exons 1-2
	5'-CGCAGGCATTGCATAGATAG-3'	NM_008093.1	Exons 4-6
	5'-CCAGACAAGAAAACGGAAGC-3'		
<i>Gata6</i>	5'-TAGGACCCCACTGAGACCAG-3'	NM_010258.3	Exon 4
	5'-CTTCCTCATCTCTGCCAAGG-3'		
	5'-CCAAGGCTCATCTCAGTGTG-3'		
<i>Hey1</i>	5'-GAGAAGGCTGGTACCCAGTG-3'	NM_010432.2	Exons 3-5
	5'-ATGCTCAGATAACGGGCAAC-3'		
<i>Hey2</i>	5'-CAGCTGCTTTCACCATCTC-3'	NM_013904.1	Exon 5
	5'-GTGGCCGAAAGAGACAGAAG-3'		
<i>Jag1</i>	5'-ATGATGGGAACCCTGTCAAG-3'	NM_013822.5	Exons 25-26
	5'-CATTGTTGGTGGTGTGTCC-3'		
<i>Mef2c</i>	5'-GTGGTTTCCGTAGCAACTCC-3'	NM_001170537.1	Exons 9-11
	5'-CAGCTGCTCAAGCTGTCAAC-3'		
<i>Notch1</i>	5'-GTCCCACCCATGACCACTAC-3'	NM_008714.3	Exon 34/3'-UTR
	5'-CCTGAAGCACTGGAAAGGAC-3'		
<i>Nrg1</i>	5'-TCCACAGCTCATCACTCCAC-3'	NM_178591.2	Exons 7-8
	5'-GGAGTCAGGGGTTTCTCTGG-3'		
<i>Rbpjκ</i>	5'-TGGTTTGGGGATGTAGAAGC-3'	NM_009035.4	Exons 10-11

	5'-GGAGTTGGCTCTGAGAATCG-3'		
<i>Tbx20</i>	5'-ACTCCTCCAGGCTCACTGAC-3'	NM_194263.2	Exons 6-8
	5'-TAGGGTGAGGAATCGGTGTC-3'		
<i>Tie2</i>	5'-TGGATTGTCACGAGGTCAAG-3'	X71426.1	Exons 17-21
	5'-CTTCTCCCTCCAGCACTGTC-3'		
